Refer to: Advances in the understanding of asthma—Medical Staff Conference—University of California, San Francisco. West J Med 130:43-55, Jan 1979

Advances in the Understanding of Asthma

These discussions are selected from the weekly staff conferences in the Department of Medicine, University of California, San Francisco. Taken from transcriptions, they are prepared by Drs. David W. Martin, Jr., Associate Professor of Medicine, and Robert C. Siegel, Associate Professor of Medicine and Orthopaedic Surgery, under the direction of Dr. Lloyd H. Smith, Jr., Professor of Medicine and Chairman of the Department of Medicine. Requests for reprints should be sent to the Department of Medicine, University of California, San Francisco, CA 94143.

DR. SMITH:* *In this Medical Staff Conference some of the newer concepts in the pathogenic mechanisms of a common disorder, asthma, will be discussed. Drs. Warren Gold and Jay Nadel of the Cardiovascular Research Institute will lead the discussion.*

DR. GOLD:† The purpose of this review is to analyze current advances in our understanding of asthma. First, we will review the biology of IgE based on studies *in vitro* and then review the roles played by mast cells, chemical mechanisms and nervous mechanisms in IgE-mediated reactions *in vivo*. Next, we will review the concept of airway hyperirritability in asthma and, finally, analyze the mechanisms controlling mucociliary clearance.

Biology of IgE

Figure 1 illustrates the general concept of IgE-mediated allergic asthma developed by immunopharmacologists from studies with isolated tissues.[1] Certain antigens, such as aeropollens, cross the bronchial epithelium and stimulate an immune response in lymphoid tissues. It is uncertain how such large molecules (10,000 to 40,000 daltons) penetrate the bronchial epithelium. One of the basic defects in asthmatic patients may be that their airways are abnormally permeable, permitting inhaled antigens to reach the lymphoid tissue, not only in the periphery, but also in the respiratory tract. The immune system elaborates a specific immunoglobulin (IgE) that has several very important characteristics: (1) IgE is present in minute concentrations in plasma.[2] (2) IgE fixes to tissues (such as mast cells in the lung and the basophils in the circulating blood). On reexposure to the same antigen, two molecules of this specific IgE-antibody react with the antigen. This reaction causes perturbation of the membrane of

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The studies by Dr. Gold on the biology of IgE were performed with D. Y. C. Yu, G.-F. Kessler, and O. L. Frick; on mast cells with G. L. Meyers, D. S. Dain, R. L. Miller, and H. R. Bourne; on 48/80 with M. R. Nisam, A. Zbinden, S. Chesrown, and D. Barnett; on cyclic nucleotides with D. Barnett, S. E. Chesrown, A. F. Zbinden, M. Nisam, B. Reed, H. R. Bourne, and K. L. Melmon; on tantalum bronchography with G.-F. Kessler, J. H. M. Austin, P. D. Graf, and G. Gamsu; and on vagus nerves with G.-F. Kessler, and D. Y. C. Yu.

The studies by Dr. Nadel on virus infections were carried out with D. M. Empey, L. A. Laitinen, L. Jacobs, L. A. Elkin, and J. Mills; on ozone with L.-Y. Lee and E. Bleecker; on ion transport with M. G. Marin, B. Davis and R. E. Oliver; on mucous gland secretion with B. Davis, M. G. Marin, I. Ueki and P. D. Graf.

ABBREVIATIONS USED IN TEXT

AMP = adenosine monophosphate
ECF-A = eosinophil chemotactic factor of anaphylaxis
GMP = guanine monophosphate
NCF = Neutrophil chemotactic factor of anaphylaxis
PAF = platelet-activating factors
R_{aw} = airway resistance
R_L = resistance to flow
SRS-A = slow reacting substance of anaphylaxis

the target cell to which the IgE molecules are bound and initiates a sequence of biochemical reactions within the cell. This results in the generation of a number of very important chemicals, or mediators of anaphylaxis. At a plasma IgE concentration of only 10 ng per ml (exceeded in 99 percent of healthy and allergic persons), the basophil is functionally saturated and is coated with 4,000 IgE molecules. When this number of IgE molecules is present, exposure of the basophil to anti-IgE causes a maximal release of mediator from the cell. Basophils from healthy subjects will release less than 25 percent of their stored mediators; basophils from allergic subjects will release more than 85 percent of their mediator content. Therefore, another important feature of the allergic state is the abnormal release of mediators.

Chemical Mediators of Anaphylaxis

Three types of mediators are illustrated in Figure 1.[1] Histamine is released in all mammalian species when IgE reactions are triggered.[2] Histamine causes smooth muscle contraction and increased permeability of the venous end of the capillary.

Slow reacting substance of anaphylaxis (SRS-A) is poorly defined chemically. It is an acidophilic lipid, not a peptide or prostaglandin, with a molecular weight of 400 daltons. There are some studies *in vitro* and *in vivo* suggesting that SRS-A produces sustained contraction of airway smooth muscle in guinea pigs and some monkeys, but not in dogs. More recently, Wanner and co-workers suggested that SRS-A inhibits mucociliary transport.

Eosinophil chemotactic factor of anaphylaxis (ECF-A) is one of a number of chemotactic factors released by the antigen-antibody interaction which attracts different cell types to the site of the immune response. ECF-A attracts eosinophils containing chemicals that inhibit IgE reactions: aryl-

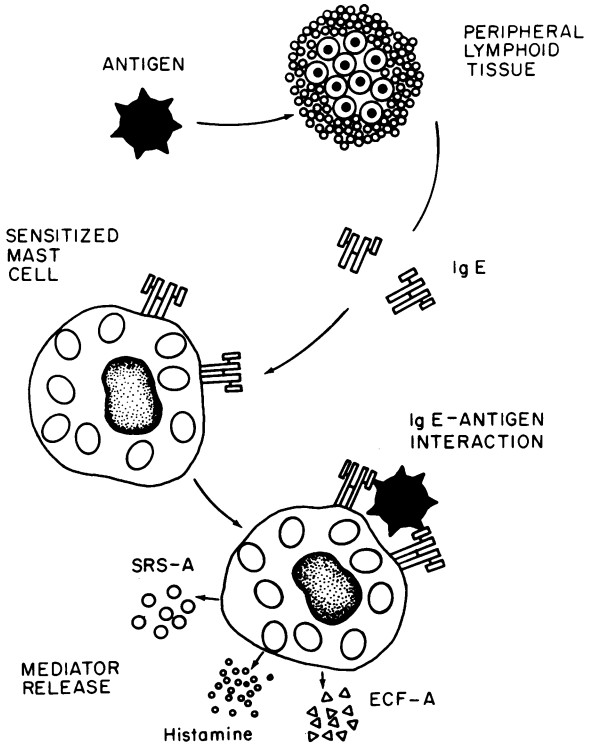

Figure 1.—Molecular mechanisms of mediator release in asthma. Antigen exposure stimulates peripheral lymphoid tissues in atopic persons to synthesize IgE. The IgE antibody becomes fixed by its Fc portion to certain cells (such as circulating basophils in the blood or mast cells in the tissues). On repeated exposure, the same antigen reacts with the antigen receptors on the Fab portion of two IgE molecules. The IgE-antigen interaction activates enzyme systems within the cell which cause the release of mediators of anaphylaxis. In lung tissue at least three mediators may be released, including histamine (bronchospasm), slow reacting substance of anaphylaxis (SRS-A, prolonged bronchospasm), and eosinophil chemotactic factor of anaphylaxis (ECF-A, eosinophil exudate). (Reproduced from Gold[1] with permission from Academic Press, Inc.)

sulfatase B inactivates SRS-A, histaminase inactivates histamine and phosphorylase D degrades certain platelet-activating factors. The action of these compounds will tend to localize or limit the severity of the reaction. Other chemotactic factors such as neutrophil chemotactic factor of anaphylaxis (NCF) attract neutrophils which may release lysosomal enzymes causing pronounced inflammation and augmentation of the reaction.[3] Finally, there are platelet-activating factors (PAF) released from the target cells which can liberate potent chemicals, such as serotonin, from platelets which may not only affect airway smooth muscle directly, but also augment the response to vagally mediated reflexes.

Modulation by Cyclic Nucleotides

Other studies *in vitro* suggest that the IgE-mediated antigen-antibody reaction is modulated by the second messengers, cyclic adenosine monophosphate (AMP) and cyclic guanine monophosphate (GMP). Figure 2 shows the effect of increasing levels of cyclic AMP by beta-adrenergic stimuli, whether by synthetic drugs administered for treatment (such as isoproterenol), or by circulating epinephrine, or the release of norepinephrine from adrenergic nerves. Each of these compounds appears to act on the beta receptor activating adenyl cyclase which synthesizes cyclic AMP. This, in turn, results in inhibition of the release of mediators by IgE.[2]

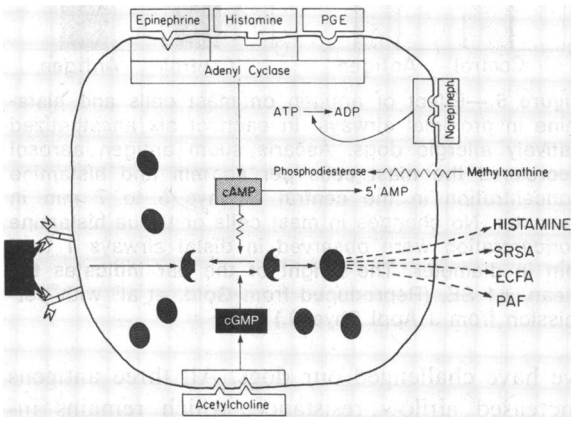

Figure 2.—Effects of endogenous hormones and mediators of inflammation on mast cells. Antigen reacts with two molecules of IgE-antibody fixed to the sensitized mast cell **(left).** This triggers an enzyme-dependent series of biochemical reactions leading to the release of mediators from the mast cell granules. These mediators may include histamine (smooth muscle contraction, increased capillary permeability), SRS-A: slow reactive substance of anaphylaxis (smooth muscle contraction and perhaps impaired mucociliary clearance), ECF-A: eosinophil chemotactic factor of anaphylaxis (attracts eosinophils to reaction site), and PAF: platelet activating factor (activates platelets leading to release of serotonin). Mediator release is augmented by increased levels of cyclic guanine monophosphate (cGMP). Increased cGMP results from stimulation by cholinergic agents administered exogenously or perhaps released during a vagal reflex response. Mediator release is inhibited by increased levels of cyclic adenosine monophosphate (cAMP). Increased cAMP results from adenyl cyclase-mediated synthesis from adenosine triphosphate (ATP). Adenyl cyclase is stimulated by endogenous hormones such as epinephrine and by mediators of inflammation such as histamine or prostaglandin (PGE). Each agent appears to have its own specific receptor site. Increased cAMP may also result from decreased catabolism if degradation by phosphodiesterase is inhibited (for example, by exogenous methylxanthine). Some work suggests that decreased cAMP may result from actions of norepinephrine (activates ATPase), resulting in augmentation of mediator release.

Thus, the release of mediators by antigen is modulated by intracellular levels of cyclic nucleotides. The complex control system permits local negative feedback terminating the reaction and localizing the response (for example, histamine or PGE effect). It also permits positive feedback to occur augmenting the reaction and leading to a generalized inflammatory response (for example, acetylcholine, norepinephrine).

Figure 2 also illustrates the action of another cyclic nucleotide, cyclic GMP. This cyclic nucleotide is increased by cholinergic stimuli and appears to augment IgE-induced release of chemical mediators. Therefore, there appears to be a "push-pull" mechanism to modulate the reactions produced by the physicochemical interaction between antigen and antibody. Kaliner and associates[4] have shown that antigen-induced release of mediators from passively sensitized human lung is altered by cyclic nucleotides: when synthetic cyclic GMP is administered, there is an increase in the amount of mediator release; conversely, when synthetic cyclic AMP is administered, there is a decrease in the amount of mediator released. Many immunopharmacologists working in this field have assumed that the same thing happens in the smooth muscle itself: that is, increased cyclic AMP is associated with relaxation of the smooth muscle; conversely, increased cyclic GMP is associated with contraction of airway smooth muscle. Although this is an appealing concept with which to analyze the action of antiasthma drugs, more recent evidence suggests that the cyclic nucleotides have much more complex ac-

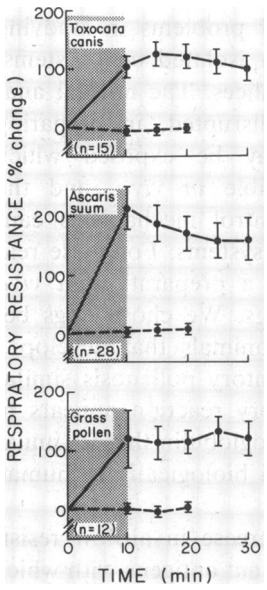

Figure 3.—Effect of different antigen aerosols on respiratory resistance in experimental canine asthma. Solid line: specific antigen aerosol. Dashed line: control aerosol containing extract to which the dog had a negative skin test. Each point represents the mean percent change in resistance ±SE. The shaded zone indicates the period of aerosol inhalation. n = number of dogs studied. (Reproduced from Gold with permission from Academic Press, Inc., Ref. 1.)

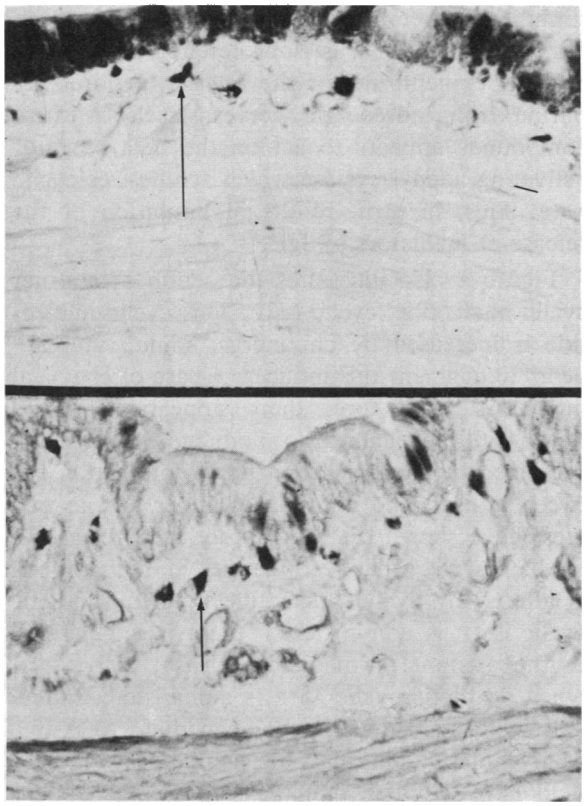

Figure 4—Mast cells in airways of natively allergic dogs. Photomicrographs of a 5-mm bronchus (bottom) and a 1-mm bronchus (top) from the lung of an allergic dog. The slide has been stained with toluidine blue which stains the granules in the mast cells (indicated by the arrows).

tions in smooth muscle and that both nucleotides may actually inhibit smooth muscle tone.

Experimental Canine Asthma

There are a number of problems in studying IgE-mediated reactions in isolated cell systems, lung fragments and lung slices. The normal anatomic relationships are disrupted, in preparing the tissues receptors may be exposed which are not normally accessible *in vivo,* and the normal neurohumoral control mechanisms cannot be studied in isolated systems. For these reasons, we have developed a preparation *in vivo* using natively allergic dogs. We chose dogs because they are the only animals that develop a naturally occurring respiratory pollenosis similar to humans. This respiratory reaction appears to be mediated by an immunoglobulin that is similar physically, chemically and biologically to human IgE.

Figure 3 shows the increase in airflow resistance caused by three different antigens with which

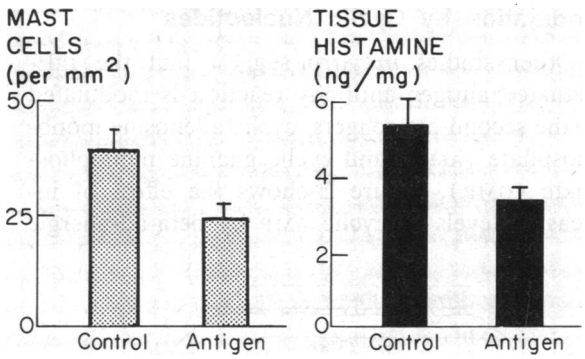

Figure 5.—Effect of antigen on mast cells and histamine in proximal airways. In each of six anesthetized natively allergic dogs, Ascaris suum antigen aerosol decreased the mast cells per sq mm and histamine concentration in the central airways 5 to 7 mm in diameter. No changes in mast cells or tissue histamine concentration were observed in distal airways 1 to 2 mm in diameter. The height of the bar indicates the mean ±1 SE. (Reproduced from Gold, et al[6] with permission from J Appl Physiol.)

we have challenged our dogs. All three antigens increased airflow resistance, which remains increased for about 30 minutes; control antigens, to which the animals had no skin reactivity, produced no airway responses.[5]

Role of Mast Cells in IgE-Mediated Reactions

The target cell of inhaled antigen in the airways of these allergic dogs is the mast cell. Figure 4 shows photomicrographs of a 5-mm bronchus (bottom) and a 1-mm bronchus (top). The slide has been counterstained with toluidine blue which is absorbed by the granules of the mast cells, permitting us to see them. The dark cells below the basement membranes are mast cells; they appear to be waiting for the antigen to cross the mucosa and reach the IgE-antibody presumably fixed to their plasma membranes. There is an inverse correlation between mast cell number (and histamine content) and bronchial diameter. Because of the close correlation between mast cell number and histamine content, we have used the release of histamine as an index of mast cell degranulation.

Physiological Effects of Antigen-Induced Release of Chemicals from Mast Cells

Figure 5 shows the effect of antigen aerosol on large airways of 5 to 7 mm in diameter in allergic dogs. The number of visualized mast cells decreased after antigen because the granules were secreted, dye was no longer taken up by the cells, and therefore the number visualized and counted

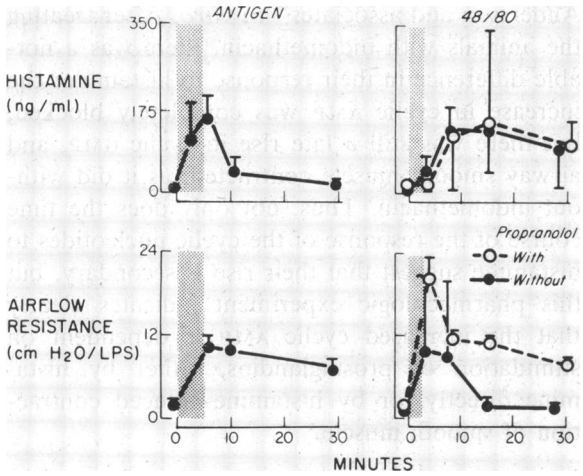

Figure 6.—Effect of antigen aerosol **(left)** and compound 48/80 aerosol **(right)** on release of histamine **(upper)** and airflow resistance **(lower).** Each point is the mean ±1SE (I) of experiments in six dogs with antigen alone (•—•), in eight dogs with 48/80 alone (•—•), and in four dogs with 48/80 during propranolol infusion (o---o). Shaded zone indicates period of aerosol inhalation. (Reproduced from Gold[7] with permission from Am Rev Resp Dis.)

decreased. This figure also shows that there is a parallel decrease in the amount of bronchial tissue histamine after antigen aerosol.[6]

As histamine was released from the tissues following antigen aerosol, it was eluted into the blood perfusing the lung, resulting in a pronounced but transient increase in arterial plasma histamine (Figure 6, left).[7] At the same time, airflow resistance following antigen challenge increased to a maximum corresponding with the release of histamine and then gradually decreased, but not nearly so quickly as the histamine decreased.[8] This suggests that other factors besides the release of stored histamine are responsible for the increased airflow resistance. In an attempt to analyze this process, we have bypassed the immunologic reaction and examined the effect of a pharmacologic agent, compound 48/80, which has been used for many years to degranulate mast cells *in vitro*. It appears to cause secretion of preformed chemicals from the mast cells in a manner similar to antigen. This permitted us to look at a portion of the sequence of reactions induced by antigen.

Physiological Effects of Pharmacological Release of Preformed Chemicals from Mast Cells

Following exposure to compound 48/80 aerosol, there was a decrease in both the mast cell number and the tissue histamine content in the airways, associated with a notable increase in the level of histamine in the plasma leaving the lung (Figure 6, right). The physiologic response to this reaction was characterized by increased airflow resistance and decreased arterial blood pressure.

These animals were treated with chlorpheniramine (which blocks H_1-histamine receptors) and then were challenged with 48/80 aerosol. Although the same amount of histamine was released, the physiologic response of airway smooth muscle was completely inhibited (data not shown). The arterial blood pressure still decreased, but this is not surprising since other workers have shown that the effect of histamine on the blood vessels involves both H_1- and H_2-receptors. These results indicate that secretion of granules from mast cells of allergic dogs results in release of stored mediator, primarily histamine, which acts on the H_1-receptor in the airway smooth muscle.

Cyclic Nucleotide Metabolism in Peripheral Canine Lung in Vivo

Many studies *in vitro* suggest that the initiation and modulation of the reaction induced by antigen-IgE antibody interaction depends on changes in cyclic nucleotide metabolism. Therefore, we have developed a method to evaluate cyclic nucleotide metabolism in our experimental dogs *in vivo* to determine what extrapulmonary and intrapulmonary mechanisms are important in regulating these chemicals. We hope to correlate the changes in cyclic nucleotide levels with changes produced in these animals by pharmacologic agents like 48/80, or by antigen, in order to understand more about the biochemical regulation of the reaction. In anesthetized dogs with open chests, we sampled tissue from within 3 cm of the edge of the lobe with clamps cooled in liquid nitrogen. Under these experimental conditions, the basal level of cyclic AMP was 88 picomoles per 100 mg of wet tissue. Cervical vagotomy did not change the cyclic nucleotide concentration. To determine if there are adrenergic influences on cyclic AMP, we infused propranolol (1 mg per kg of body weight, intravenously) and observed a pronounced decrease in the level of cyclic AMP. This result suggests that the basal level of cyclic AMP in this part of the lung is stimulated by catecholamines. When we sectioned the sympathetic nerves to the lung, there was no change in cyclic AMP concentration; therefore, the effect of propranolol does not appear to result

from blockade of a sympathetic nervous effect on the basal level of cyclic AMP. Another possibility is that propranolol unmasks an alpha-adrenergic effect on cyclic AMP. Therefore, we infused propranolol and then administered an alpha antagonist (phentolamine), but this did not change the level of cyclic AMP. This suggests that the basal level of cyclic AMP in the lung must be slightly increased under our experimental conditions due to a circulating catecholamine, presumably released from the adrenal gland, stimulating beta-adrenergic receptors in the periphery of the lung.[7]

Effect of Histamine on Cyclic Nucleotide Metabolism

Histamine (0.05 mg per kg of body weight, given intravenously), an important mediator of anaphylaxis, was infused into the periphery of the lung through the pulmonary artery. Histamine caused a pronounced increase in both cyclic AMP and cyclic GMP; saline control infusion caused no changes in cyclic nucleotides. The increase in nucleotides occurred approximately two minutes after histamine was injected; however, airway smooth muscle contracted within 20 seconds after the bolus of histamine was infused *in vivo*. Clearly, changes in cyclic nucleotides must be secondary events. This conclusion is substantiated further by studies of the mechanism of the response to histamine. The usual response to histamine administered as a bolus intravenously is an increase of both cyclic nucleotides, a contraction of airway smooth muscle indicated by the increased total lung resistance and decreased static lung compliance, and a decrease in arterial blood pressure. When an antihistamine (chlorpheniramine, an H_1-antagonist) was administered before the histamine, there was a qualitative change from the usual response: both the increase in cyclic nucleotide levels and contraction of airway smooth muscle were prevented. The effect on smooth muscle in the cardiovascular system was only partially inhibited because, as already mentioned, this reaction to histamine depends on H_2-receptors, as well as H_1-receptors. We also examined the effect of indomethacin on this reaction. We chose indomethacin because previous workers have suggested that contraction of smooth muscle in the lung causes the release of prostaglandins; we speculated that the secondary rise in cyclic nucleotides was related to the contraction of smooth muscle induced by histamine, as suggested by Stoner and co-workers[9] and Andersson and associates[10] *in vitro*. After treating the animals with indomethacin, there was a notable difference in their response to histamine: the increase in cyclic AMP was completely blocked, but there was still a late rise in cyclic GMP, and airway smooth muscle contracted, as it did without indomethacin. Thus, not only does the time course of the response of the cyclic nucleotides to histamine suggest that their rise is secondary, but this pharmacologic experiment indicates clearly that the increased cyclic AMP is dependent on stimulation of prostaglandins, either by histamine directly, or by histamine-induced contraction of smooth muscle.[7]

Nervous Mechanisms in IgE-Mediated Reactions

In addition to these biochemical mechanisms involved in regulating the respiratory response to antigen, there are also important neural mechanisms involved. DeKock and associates[11] showed that when histamine was injected directly into the bronchial circulation of dogs, there was a progressive increase in airflow resistance. When the same experiment was done with conduction in the vagus nerves blocked, the reaction to histamine was notably inhibited. There was a direct, local effect, indicated by the 100 percent increase in resistance induced by 500 μg of histamine when the vagi were blocked. But in the presence of intact vagus nerves, there was an eightfold amplification of the reaction to the same dose of histamine. Therefore, we studied the role of the vagus nerves in modulating the response to antigen in our allergic dogs. Our hypothesis was that one amplification system of the antigen-antibody reaction is chemical and involves direct local effects on airways; another amplification system involves reflex mechanisms in vagal cholinergic pathways. Figure 7 is a tantalum bronchogram in which the airways were visualized with an inert radiopaque dust and the lungs were divided by a Carlen catheter. When we delivered antigen to the left lung only, not only did the left lung constrict, but the right lung constricted as well. Thus, bilateral constriction of the airways resulted when we challenged one lung only.[12] The effect of blocking the left vagus innervating the lung which received the antigen is shown in Figure 8. After antigen was delivered to the left lung, airflow resistance increased not only in the left lung but also in the right lung. When we cooled the left vagus nerve, blocking afferent signals conducted in it, the increased resistance was inhibited not

only in the left lung but also in the right lung. When we rewarmed the left vagus nerve, the action recurred bilaterally. We conclude that the antigen-antibody reaction in the left lung released mediator which stimulated sensory nerve endings in the left vagus, sending afferent signals to the central nervous system and efferent signals were transmitted in the right vagus nerve, producing the reaction observed in the right lung. The local, direct effects of mediators are indicated by the height of the bars during vagal cooling; the neural amplification by the height of the bars when the vagus nerves are both at 37°C.

Summary

The reaction immunologically mediated by antigen and IgE-antibody involves mast cells and their release of mediators (see Figure 9). These include preformed chemicals such as histamine, and the initiation of the synthesis of unstored mediators, such as slow-reactive substance, which can have direct, local effects on the airway smooth muscle. In addition to this local effect, sensory nerve endings within the airway are stimulated with afferent signals carried in the vagus nerves to the central nervous system and efferent signals carried in the vagus nerves to the smooth muscle, resulting in further amplification of the reaction. Our present experiments are designed to examine the effect of modulating this process by vagal-parasympathetic mechanisms as well as by sympathetic nervous and circulating humoral mechanisms. These experiments should clarify many of the biochemical and nervous mechanisms that

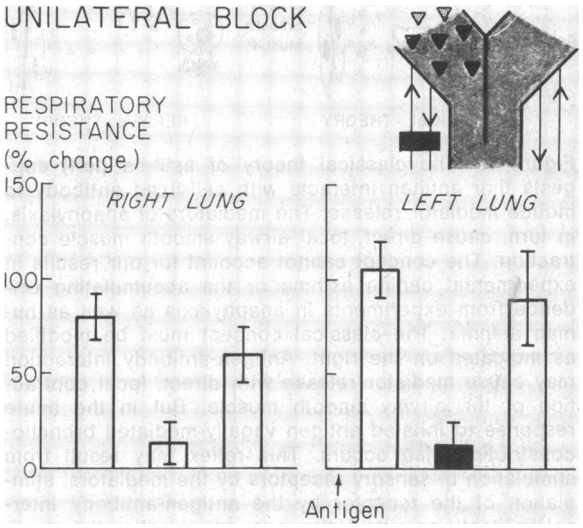

Figure 8.—Effect of ipsilateral vagal blockade on bilateral bronchoconstriction induced by unilateral antigen inhalation. Antigen aerosol was administered to the left lung only as indicated by the triangles in the diagram and the arrow in the right lower panel. Respiratory resistance is expressed in percentage change from control. The height of the bar indicates the mean response ±SE (I) in five dogs. Open bars: vagus nerve at 37°C before and after cooling left vagus nerve. Solid bar: left vagus nerve at 0°C. (Reproduced from Gold[1] with permission from Academic Press, Inc.)

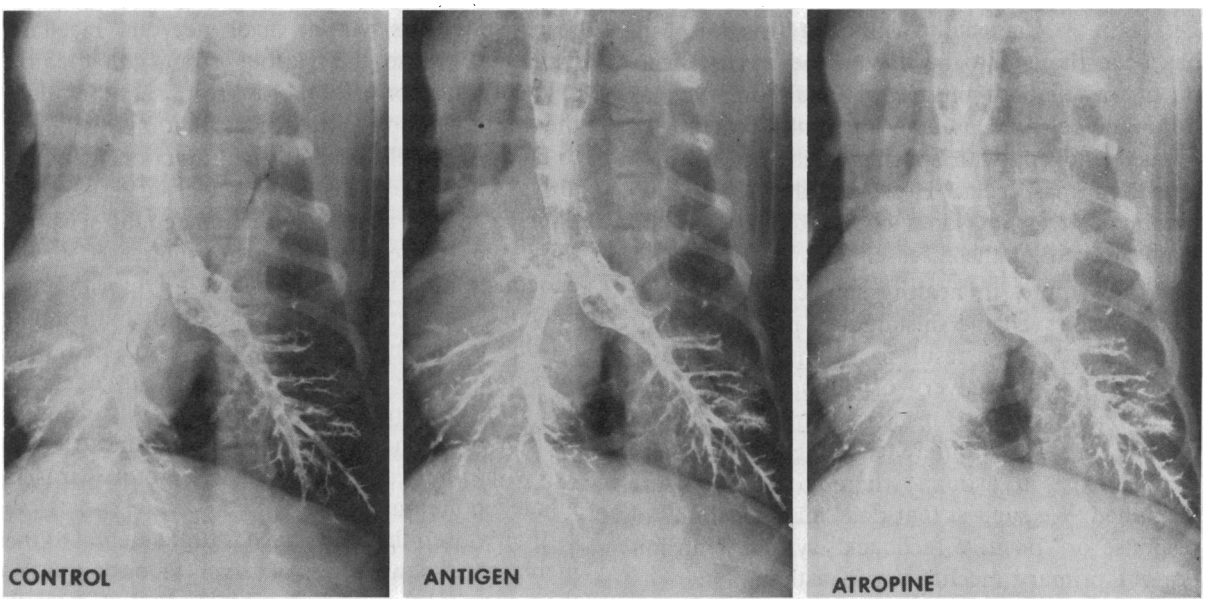

Figure 7.—Tantalum bronchograms showing effect of unilateral antigen inhalation. Control, localized bulges in walls of trachea and left main bronchus can be seen due to balloons on the Carlen catheter. After antigen aerosol inhalation by the left lung only, bilateral bronchoconstriction occurred. After atropine sulfate given intravenously, bronchoconstriction was inhibited in both lungs. (Reproduced from Gold, et al[12] with permission from J Appl Physiol.)

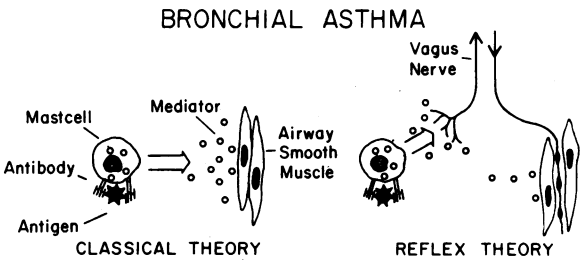

Figure 9.—The classical theory of asthma **(left)** suggests that antigen interacts with cell-fixed antibody to induce mediator release. The mediators of anaphylaxis, in turn, cause direct, local airway smooth muscle contraction. The concept cannot account for our results in experimental canine asthma or the accumulating evidence from experiments in anaphylaxis as well as human asthma. The classical concept must be modified as indicated on the right. Antigen-antibody interaction may cause mediator release with direct, local contraction of the airway smooth muscle. But in the acute response to inhaled antigen vagally-mediated bronchoconstriction also occurs. This reflex may result from stimulation of sensory receptors by the mediators, stimulation of the receptor by the antigen-antibody interaction itself, or stimulation secondarily after the mediators cause smooth muscle spasm and deformation of the receptors. Whatever the mechanism, it is clear that the parasympathetic nervous system is of central importance in the acute bronchoconstrictor response to inhaled antigen. (Reproduced from Gold[1] with permission from Academic Press, Inc.)

modulate the reaction between antigen and IgE-antibody.

Airway Hyperirritability

DR. NADEL:* Classically, asthmatic bronchospasm has been thought to be due to the direct effects on airway smooth muscle of mediators such as histamine released as a result of the reaction of inhaled antigens with specific antibodies fixed to sensitized mast cells. However, this simple explanation of asthma does not adequately explain many aspects of the disease.

For example, in healthy subjects, delivery of mediators (such as histamine, prostaglandins, serotonin) does not result in asthmatic-type bronchoconstriction. Delivery of the same mediators to asthmatic subjects results in dramatic airway narrowing.[13] These differences suggest that mechanisms exist in asthma whereby responses are magnified. We suggest that this "magnification" of response or "positive feedback" system is an important primary mechanism in asthma.

Further evidence that immunologic release of bronchoactive mediators is an inadequate explanation for the clinical findings in asthma derives from observations in allergic patients: Patients with atopic rhinitis may respond with bronchoconstriction to inhaled aerosols of specific antigen but do not have clinical evidence of asthma. We suggest that the presence or absence of asthma in these allergic patients is determined by whether the "positive feedback" system exists in the airways. Even in allergic ("extrinsic") asthmatic patients, clinical attacks usually correlate poorly with exposure to allergens. Moreover, many adult asthmatic patients have no evidence of allergic disease (hence the name intrinsic asthma). In patients with both extrinsic and intrinsic asthma, clinical attacks are usually triggered by nonantigenic stimuli, such as cold air, inhalation of irritating chemicals and dusts, exercise, and respiratory maneuvers (such as laughing, coughing, crying, hyperventilation). The tendency of patients with asthma to develop bronchoconstriction to a greater extent in response to smaller doses of various stimuli (including bronchoactive mediators) is an important characteristic of all asthmatic patients. The fact that the increased responsiveness to cold air, respiratory maneuvers, aerosols of histamine and citric acid,[13] aerosols of methacholine,[14] prostaglandin $F_{2\alpha}$,[15] and aerosols of surfactant and propellant[16] is abolished by atropine and by other anticholinergic drugs indicates that cholinergic postganglionic pathways are involved. We have suggested that the exaggerated response occurs via a vagal nervous mechanism.[13,17] Because the various stimuli that cause an increased bronchomotor response stimulate the rapidly-adapting receptors that are believed to lie immediately beneath tight junctions between airway epithelial cells,[18] we have suggested that damage to the airway epithelium could sensitize these sensory receptors, causing increased bronchoconstriction via a vagal reflex.[13,17] In asthmatic patients, damage to the airway epithelium might be due to inflammatory changes associated with an immunologic response (such as in extrinsic asthma), an infectious process (for example, "asthmatic bronchitis") or could be due to damage with other causes (such as respiratory viruses, inhaled pollutants[19,20]).

If airway epithelial damage is fundamental to the increased responsiveness of airway smooth muscle, then even subtle and reversible epithelial damage might cause a transient increase in bronchomotor responsiveness in otherwise healthy subjects. Damage to the airway epithelium can be produced

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by relatively low concentrations of ozone.[21,22] Anatomic studies indicate that the damage to the airways is maximal in 24 hours, and the airway epithelium heals within one to two weeks. Therefore, we exposed five dogs to ozone (0.7 to 1.2 ppm for two hours), and studied the effect of inhaled histamine sulfate aerosol (2 percent solution; five breaths delivered from a DeVilbiss #40 nebulizer). We evaluated the effect of histamine on the airways by measuring total pulmonary resistance to airflow (R_L). In each of the five dogs 24 hours after ozone exposure, the baseline R_L was unchanged, but the increase of R_L caused by histamine aerosol was greater than in the control state (Figure 10). This transient state of increased bronchial responsiveness was maximal 24 hours after exposure to ozone (the time when evidence of anatomic damage was greatest) and returned to control levels gradually over 7 to 28 days.[23] To test our hypothesis that cholinergic pathways were involved in the ozone effects, we pretreated the dogs with atropine sulfate aerosol (1.5 percent solution; ten breaths). Atropine did not affect the baseline R_L significantly, either before or after ozone exposure, but abolished the increased bronchomotor response to histamine after exposure to ozone (Figure 11, upper panel). Since

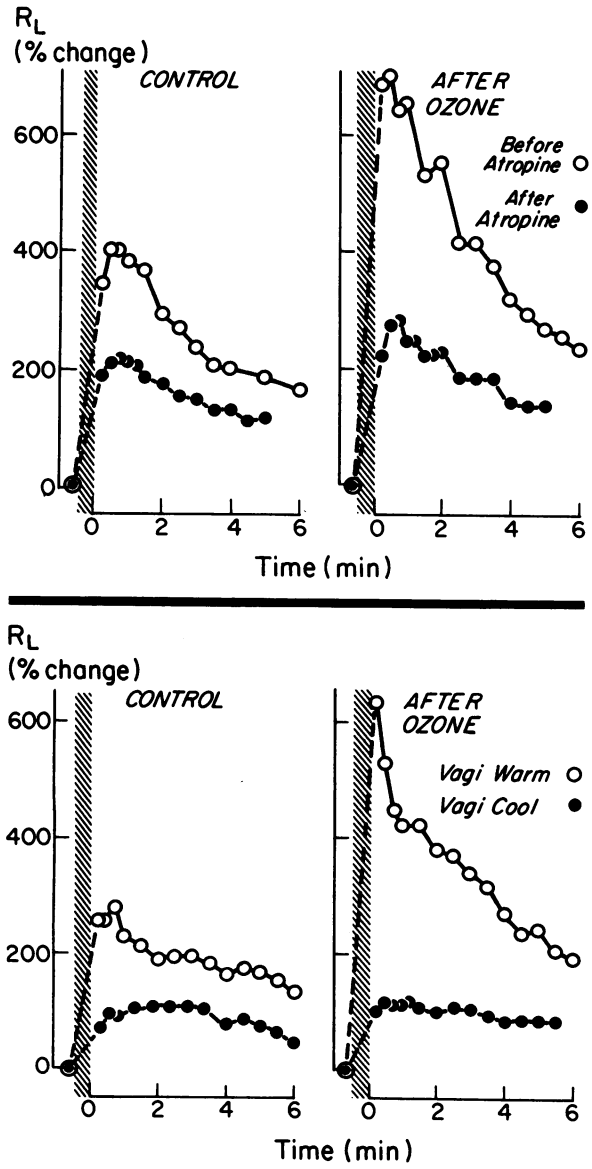

Figure 10.—Effect of inhaled histamine aerosol (five breaths of a 2 percent solution) (shaded bar) on total pulmonary resistance (R_L) in one dog, before ozone **(left)** and after ozone **(right).** Each point is the mean of three to five determinations. (o—o) = before intervention. **(Upper panel)** (•—•) = After inhalation of atropine sulfate aerosol (ten breaths of a 1.5 percent solution). **(Lower panel)** (•—•) = During cooling blockade of the cervical vagus nerves to −2°C. (Reproduced from Nadel[33] with permission from Am Rev Resp Dis.)

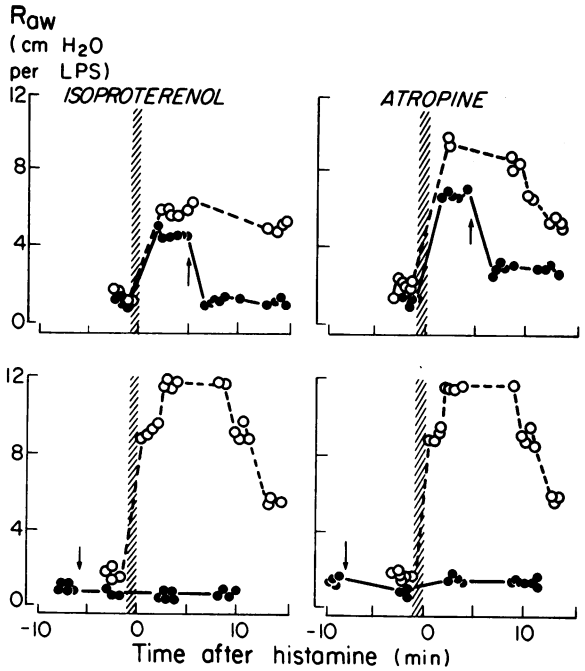

Figure 11.—Effects of isoproterenol (0.5 percent, one breath; left panels) and atropine (0.2 percent, 20 breaths; right panels) on airway resistance (R_{aw}) after inhalation of histamine aerosol (1.6 percent, ten breaths; shaded bars) by human subjects with colds. Each panel shows the effect of histamine alone (o) and after administration of isoproterenol or atropine (at the arrows, •) in the same subject. Upper panels show the effects of isoproterenol and atropine in reversing the effects of histamine; lower panels show the same drugs preventing the responses to histamine. The arrows indicate the time at which atropine or isoproterenol was administered. (Reproduced from Empey[19] with permission from Am Rev Resp Dis.)

the vagus nerves provide the cholinergic innervation of the airway smooth muscle, we studied the effect of blocking conduction in the vagus nerves. Cooling blockade of vagal nervous conduction abolished the increased bronchomotor response to histamine after ozone exposure (Figure 11, lower panel).

Histamine has two effects on bronchomotor tone. It can act locally on airway smooth muscle to cause contraction;[24] it can also stimulate vagal sensory receptors in the airways and cause reflex bronchoconstriction.[11] Since the increased responsiveness of the airways to inhaled histamine after ozone exposure was abolished by atropine and by blocking conduction in the vagus nerves, the increased response was due to potentiation of the reflex, not the local response.

Because viral respiratory tract infections also cause transient damage to the airway epithelium, we studied the effect of inhalation of histamine aerosol (1.6 percent solution; ten breaths) on airway resistance (R_{aw}) in otherwise healthy subjects with colds.[19] In 16 normal subjects with colds, baseline R_{aw} was not significantly different from eleven healthy control subjects. However, inhalation of histamine aerosol produced a greater increase in R_{aw} in the subjects with colds compared to the control group. The increased bronchial responsiveness appeared within two or three days after the onset of the cold and returned to normal in variable periods up to seven weeks. Because of the difficulties in timing the onset of spontaneous viral infections and in determining their exact causes, we also studied in a double-blind manner the effect of live attenuated influenza virus (Alice strains of influenza A and B virus; Smith Kline & French) delivered into the airways. We monitored bronchial response to inhaled histamine in healthy subjects with hemagglutination-inhibition antibody titers of 1:8 or less to homologous influenza A virus. Subjects who received placebo showed no change in the response to inhaled histamine aerosol, but subjects who received virus and who subsequently developed greater than four-fold rises in hemagglutination antibody titers showed increased bronchomotor responses to inhaled histamine compared to their control states. Bronchial responsiveness was maximal on the second day and disappeared by the ninth day after administration of virus. Atropine sulfate aerosol (0.2 percent solution; 20 breaths) abolished the increased bronchomotor response to histamine in subjects with spontaneous colds (Figure 11) and in subjects inoculated with live attenuated influenza virus.[25]

Exposure to ozone and respiratory viral infections both cause transient damage to the airway epithelium, and we suggest that this damage is the cause of the transiently heightened bronchial responses to inhaled histamine aerosols. The fact that, in each case, the increased responses were abolished by blockade of conduction of the vagus nerves and by atropine sulfate (a drug whose major effect is to block postganglionic cholinergic pathways) is compatible with our hypothesis that damage to the airway epithelium exposes sensory nerve endings and potentiates reflex bronchomotor responses.

Stimulation of sensory nerve endings in the conducting airways causes reflex bronchoconstriction, but these sensory pathways also have other central nervous connections and thus cause other reflex responses (such as cough, rapid, shallow breathing).[26] Thus, the threshold concentration of an inhaled irritant (citric acid aerosol) required to produce cough decreased during respiratory viral infection,[19] and this provides further evidence that sensitization of nerve endings occurs when airway epithelial damage occurs. The rapid, shallow breathing pattern observed after inhalation of ozone[27] may also be due to oxidant damage to the airway epithelium with subsequent effects on vagal nerve endings and reflex stimulation of ventilation. In fact, the chronic respiratory alkalosis in asthma[28] and even the sensation of dyspnea perceived by asthmatic patients may be due to abnormal stimulation of nerve endings in the airway epithelium.

Studies of the pathogenesis of asthma have concentrated on the airway smooth muscle and on mast cells. Our hypothesis is that damage to the airway epithelium plays an important primary role in asthma. Therefore, we suggest that: (1) In atopic patients in whom bronchoconstriction develops following inhalation of specific antigen, the presence or absence of clinical bronchospastic disease may depend on the presence of airway epithelial damage. (2) In intrinsic asthma, mediators may be released from cells other than mast cells (for example, leukocytes, macrophages, platelets). In this case (as with extrinsic asthma), epithelial damage sets up the "positive feedback" system that allows clinical bronchospasm to become evident. (3) Our studies on respiratory viruses in healthy subjects provide a mechanism

by which viral infections precipitate asthmatic attacks[29,30] and cause exacerbations of chronic bronchitis.[31] (4) Ozone has reached ambient levels as high as 0.54 ppm in Los Angeles and in other cities. Consequent damage to the airway epithelium that occurs at these concentrations[21] could affect airway function deleteriously, especially in patients with pulmonary diseases (such as asthma). (5) Mucous glands receive cholinergic innervation,[32] and application of cholinergic agonists to the airways stimulates secretion.[33] The mucous hypersecretion that may occur in asthmatic patients could be due to cholinergic reflex pathways (potentiated by sensitization of the sensory pathways). Both smooth muscle and mucous gland hypertrophy that occur in asthma and in chronic bronchitis may be due to chronic reflex overstimulation.

Mucociliary Clearance

In some asthmatic patients progressive bronchial narrowing develops, relatively unresponsive to bronchodilator drugs. Some investigators have suggested that this narrowing is due to continued smooth muscle contraction that has, by continued use, become resistant to beta-adrenergic drugs. However, postmortem examination of the airways in such patients shows there to be obstruction of airways due to inspissation of mucus. Respiratory tract fluid is secreted into the airway lumen and then propelled up the airways by ciliary action. Clearance of inhaled insoluble particulate material is determined by the interaction of ciliary motility and the physical properties of the mucous blanket. Water lubricates the airways and facilitates coupling between mucus and cilia by forming a layer through which the cilia move to propel mucus toward the mouth. Abnormal clearance from the airways in disease (such as in status asthmaticus and cystic fibrosis) may be due to a loss of normal water flow into the airway lumen. Since water flow is linked to active ion transport in other epithelia, we set out to determine whether such active transport of ions exists in the airway epithelium. To accomplish this, we applied Ussing's short-circuit technique to canine airway epithelium *in vitro*.[34] We anesthetized dogs, excised the trachea, dissected off the trachealis muscle and mounted the posterior membranous portion of the remaining epithelium between the two halves of an Ussing chamber. We measured electrical potential difference and short-circuit current via two electrical circuits connected to the chamber by agar bridges. We used radioisotopes (^{22}Na, ^{24}Na, ^{36}Cl) to measure unidirectional ion fluxes. We found a stable potential difference (30.7 ± 1.7 mV) lumen negative to submucosa; and a stable short-circuit current (108 ± 8 μA per sq cm). These findings suggested the presence of an active ion pumping mechanism across the airway epithelium. We confirmed this by finding a net flux of Cl^- toward the lumen (2.7 ± 0.6 μEq per sq cm per hour) and a net flux of Na^+ toward the submucosa (0.8 ± 0.2 μEq per sq cm per hour).

This net transport of ions toward the airway lumen could provide a mechanism for regulating water flow. Because furosemide inhibits Cl^- transport in the thick ascending limb of the nephron, we studied the effect of this drug on Cl^- transport across the airway epithelium.[35] Furosemide inhibited Cl^- transport when the drug was added to the submucosal side, but not when it was added to the luminal side of the epithelium. These findings suggest that the Cl^- pump is located on the submucosal side of the epithelium.

Several pharmacologic agents important in asthma have significant effects on epithelial ion transport in airways. Histamine, a mediator of the allergic response of airways, increased the net ion flux of Cl^- toward the lumen, a response that was inhibited by diphenhydramine, but not by burimamide, suggesting that this effect of histamine is via a H_1-receptor.[36] Acetylcholine, the mediator released at postganglionic vagal nerve endings, also increased net flux of Cl^- toward the lumen,[37] and this provides a mechanism whereby water secretion could occur in response to cholinergic stimulation. Hypersecretion may be a part of the reflex that occurs in response to mechanical, chemical and pharmacological stimulation of the airways in asthma. Beta-adrenergic agonists are known to have beneficial therapeutic effects in asthma. In addition to their bronchodilator effects, these agents increase Cl^- flux toward the airway lumen,[38] and this effect may be the mechanism whereby mucociliary clearance is improved.[39] Our studies have shown an active ion transport mechanism whereby water transport may be regulated normally in the airway epithelium. Inhibition of ion transport may occur in some asthmatic patients and may lead to abnormal mucociliary clearance and inspissation of mucus that occurs in status asthmaticus. Drugs affecting ion transport may provide a rational basis for therapy in these patients.

Airway epithelium is made up of multiple types of cells (such as ciliated cells, goblet cells, mucous gland cells). A study of regulation requires isolation of each of these biological subunits. Because of their size, submucosal glands are likely to be major contributors to total airway secretion, and we are developing techniques for their study.[33] In anesthetized dogs, we incised the anterior surface of the trachea, pulled the edges widely apart to expose the epithelial surface which we visualized through a dissecting microscope. Since no landmarks exist to locate the gland duct openings, we deposited a fine layer of powdered tantalum on the surface to prevent the normal dispersion of fluid on the surface. Anatomic studies suggest that cholinergic nerves innervate the glands.[32] When we stimulated the vagus nerves electrically, elevations appeared in the tantalum layer due to accumulation of submucosal gland fluid (Figure 12). We proved that the elevations were located above mucous gland duct openings by fixing the tissue and gently removing the elevations: Duct openings were present over each removed elevation (Figure 13). We can now remove fluid from individual gland ducts with micropipettes and can study their regulation by techniques analogous to study of the nephron. These techniques should allow a better understanding of the role of the mucous glands in the regulation of normal airway secretions and the effects of various interventions.

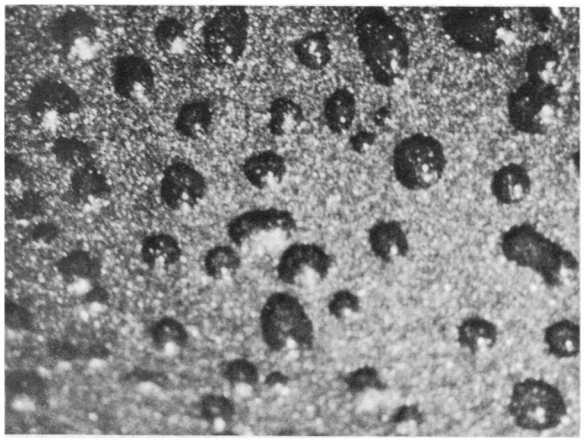

Figure 12.—Photograph of exposed canine cervical tracheal epithelium obtained through a dissecting microscope. The epithelium was coated with powdered tantalum, and the cervical vagus nerves were stimulated to produce elevations (hillocks) due to accumulated secretions. (Reproduced from Nadel[33] with permission from Am Rev Resp Dis.)

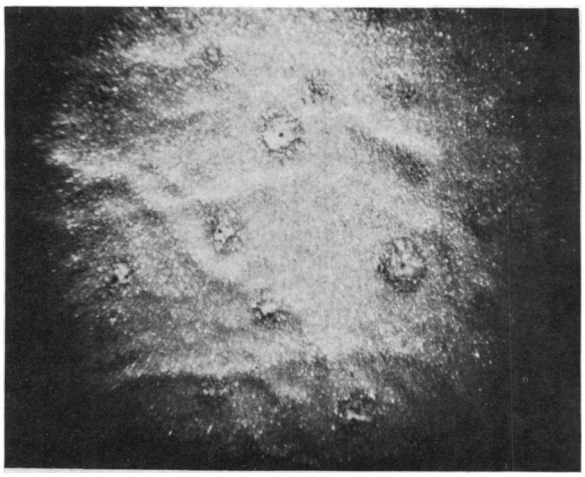

Figure 13—Photograph of gland duct openings in exposed canine epithelium obtained through a dissecting microscope. After the tracheal epithelium was coated with powdered tantalum and the vagus nerves were stimulated to produce secretory elevations, the trachea was fixed with Bouin solution, and hillocks were removed with a fine cotton swab. Photograph shows gland duct openings (black dots, approximately 30 mm in diameter) surrounded immediately by a circular area (approximately 300 μm) of removed secretions. Dark intervening surface of tracheal epithelium is covered with tantalum powder.

Summary

In this review we have summarized current concepts in the biology of IgE, the roles played by mast cells, chemical and nervous mechanisms involved in IgE-mediated reactions, a new hypothesis explaining airway hyperirritability and, finally, mechanisms controlling mucociliary clearance in airways. We believe that our understanding of the pathogenesis of asthma will be improved by these advances, but also that they will provide a basis for developing improved therapy of patients with this disease.

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